09/807,507

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Frank C. Eisenschenk

Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322 AND UNDER 37 CFR 1.323
Docket No. G-052US02PCT
Patent No. 7,011,942

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Dorra Cherif
Issued	:	March 14, 2006
Patent No.	:	7,011,942
For	:	Fluorescent Probes for Chromosome Painting

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322 (OFFICE MISTAKE) AND UNDER 37 CFR 1.323 (APPLICANT'S MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Title page, Item (54) Title: "Chromosomal"	Page 1, Title: --Chromosome--
Patent Reads:	**Application Should Read:**
Column 5, Line 25: "Richard P"	Page 7, Line 25: --Richard P.--

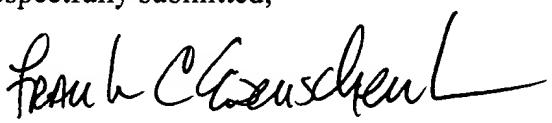
Column 6, Lines 17-18: "optical filteres"	Page 9, Line 4: --optical filters--
Column 9, Line 14: "Richard P"	Page 14, Line 2: --Richard P.--
Patent Reads:	**Application Reads:**
Column 14, Line 13: "5-bromodeoxyunridine"	Page 23, Line 2: --5-bromodeoxyuridine--
Patent Reads:	**Application Should Read:**
Column 17, Line 8: "SabileA."	Page 28, Line 29: --Sabile A.--
Patent Reads:	**Application Reads:**
Column 17, Line 42: "613 μm"	Amendment dated June 30, 2005 (original claim 11, renumbered as claim 1): --613 nm--
Column 18, Line 42: "670 μm"	Amendment dated June 30, 2005 (original claim 27 , renumbered as claim 2: --670 nm--.

A true and correct copy of the Amendment dated June 30, 2005 and pages 1, 7, 9, 14, 23, and 28 of the specification as filed which supports Applicant's assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

The Commissioner is authorized to charge the fee of $100.00 for the amendment to Deposit Account No. 19-0065. The Commissioner is also authorized to charge any additional fees as required under 37 CFR 1.20(a) to Deposit Account 19-0065. Two copies of this letter are enclosed for Deposit Account authorization.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



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FCE/gyl

Enclosures: Certificate of Correction
Copy of Amendment dated June 30, 2005
Copy of pages 1, 7, 9, 14, 23, and 28 of specification

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,011,942

APPLICATION NO.: 09/807,507

DATED : March 14, 2006

INVENTOR : Dorra Cherif

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,
Item (54), Title,
"Chromosomal" should read --Chromosome--.

Column 5,
Line 25, "Richard P" should read --Richard P.--.

Column 6,
Lines 17-18, "optical filteres" --optical filters--.

Column 9,
Line 14, "Richard P" should read --Richard P.--.

Column 14,
Line 13, "5-bromodeoxyunridine" should read --5-bromodeoxyuridine--.

Column 17,
Line 8, "SabileA." should read --Sabile A.--
Line 42, "613 μm" should read --613 nm--.

Column 18,
Line 42, "670 μm" should read --670 nm--.

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PATENT NO. 7,011,942

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,011,942

APPLICATION NO.: 09/807,507

DATED : March 14, 2006

INVENTOR : Dorra Cherif

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"Chromosomal" should read --Chromosome--.

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Column 17,
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Column 18,
Line 42, "670 μm" should read --670 nm--.

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APR 27 2006

FLUORESCENT PROBES FOR CHROMOSOME PAINTING

The present invention relates to chromosome painting and more particularly the fluorescent probes which can be used in methods such as the FISH ("Fluorescence *In Situ* Hybridization") method. The invention also relates to combinations of fluorophores and of optical filters.

In situ hybridization is a technique which makes it possible to detect a DNA (or RNA) sequence by means of a probe having a specific sequence which is homologous to that studied. It is based on the complementarity of the nucleotides (A/T, A/U, G/C) and it can be carried out under precise physicochemical conditions on chromosome or tissue preparations. The result of the *in situ* hybridization process is the formation of a hybrid between a probe and a target. *In situ* hybridization includes a denaturing step and also a step for detecting the hybrid or the probe which is carried out after the *in situ* hybridization of the probe to the target. The sample may adhere in the form of a layer to the surface of the slide and this sample may, for example, comprise or contain individual chromosomes or chromosomal regions which have been treated in order to maintain their morphologies under denaturing conditions. In the context of fluorescence *in situ* hybridization, the probes are labeled with a fluorophore and the hybridization is revealed by fluorescent labeling.

The recent development of this technique allows the simultaneous visualization, on the same preparation, of several probes each revealed by a different fluorophore. This technique, called multicolor FISH or multi-FISH, has been made possible by the combination of filters specific for the wavelengths of emission of the different fluorescent molecules ensuring the labeling with the aid of a computer-aided imaging carried out by means of infrared-sensitive high-resolution cold CCD cameras (Schröck et al., 1996; Speicher et al., 1996).

The use of probes having a specific sequence homologous to a precise chromosomal sequence or a whole chromosome coupled with the potential for a multicolor fluorescent labeling makes it possible to develop so-called chromosome painting techniques, that is to say to obtain chromosomes of different colors and thus to obtain, if desired, a multicolor complete karyotype. Karyotype is understood to mean the characteristic arrangement of the chromosomes of a cell in the

present invention. Preferably, the primers specific for the Alu DNA sequences consist of the SR1 primer whose sequence is described in SEQ ID No 1 and the primer specific for the LINE DNA sequence is preferably the L1H primer whose sequence is described in SEQ ID Nos 2 and 3.

Alternatively, probes according to the present invention may also be derived from a mixture of two IRS-PCR amplification products which is composed of:

- PCR amplification product obtained using the primer specific for the LINE DNA sequences,

- PCR amplification product obtained using the primer specific for the Alu DNA sequences and the primer specific for the LINE DNA sequences.

The present invention also relates to a method of producing probes intended for labeling human chromosomes, characterized in that said method comprises the mixing of two amplification products obtained by two IRS-PCR amplifications from said chromosomes using on the one hand PCR primers specific for the Alu and LINE DNA sequences, and, on the other hand, PCR primers specific for the LINE DNA sequences.

The present invention also comprises the use of fluorophores and of filters whose combination makes it possible to ensure a chromosome painting providing very readable karyotypes, that is to say to obtain contrasted and well-defined chromosome paint colors.

Thus, the DNA probes described above are labeled directly or indirectly by fluorescence techniques. Non-exhaustively, the fluorophores used for the labeling may be chosen from markers of the cyanine, rhodamine, fluorescein, Bobipy, Texas Red, Oregon Green, Cascade Blue type. In particular, all the fluorophores cited in "Handbook of fluorescent probes and research chemicals" (Richard P Haugland, 1996, Molecular Probes, MTZ Spence Ed., more particularly p 145-146, 153, 155-156, 157-158, 161) can be used to label the probes of the present invention.

Preferably, the probes according to the invention are labeled with at least 1, 2, 3, 4 or 5 fluorophores chosen from the following group: fluorescein isothiocyanate (FITC), Texas Red (TR for Texas Red), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7), Bodipy 630/650.

The preferred method for labeling the DNA probes is "Nick translation".

The present invention also relates to a multicolor FISH method intended for studying the karyotype, characterized in that the DNA probes are labeled with fluorophores and in that each fluorophore having a specific absorption and emission wavelength is combined with a pair of optical filteres, one for absorption and one for emission.

The fluorophores are chosen such that the overlapping of the absorption and emission spectra between the different fluorophores is minimal. More particularly, it is important that there is no overlapping between the absorption and emission maxima of the different fluorophores.

Each fluorophore is used with a pair of optical filters; one for absorption and one for emission. The filters make it possible to select the passbands such that the wavelengths corresponding to an overlap with another fluorophore are eliminated. Accordingly, the filters used in the present invention are preferably of the narrow passband optical filter type. The filters are preferably of a superior quality because it is important that the filter does not allow light outside the passband to pass through.

Preferably, the present invention also relates to a multicolor FISH method intended in particular for studying the karyotype, characterized in that the DNA probes according to the present invention are labeled with fluorophores and in that each fluorophore having a specific absorption and emission wavelength is combined with a pair of optical filters, one for absorption and the other for emission, said method using fluorophores and pairs of filters chosen from the following group:

a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm combined with an excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical),

b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical),

c) the fluorophore TR having a maximum absorption wavelength of 593 nm

transduction, pH and ion concentrations (for example calcium, potassium, magnesium and zinc concentrations) (Richard P Haugland, 1996, Molecular Probes, MTZ Spend Ed.). It may allow the study of expression and translation.

The invention therefore also relates to a combination of fluorophores chosen from: fluorescein isothiocyanate (FITC), Texas Red (TR for Texas Red), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7), Bodipy 630/650.

Preferably, the combination of fluorophores comprises at least 2, 3, 4, 5, 6 or 7 fluorophores chosen from: fluorescein isothiocyanate (FITC), Texas Red (TR for Texas Red), cyanine 3 (Cy3), cyanine 5 (Cy5), cyanine 5.5 (Cy5.5), cyanine 7 (Cy7), Bodipy 630/650.

A combination of preferred fluorophores of the present invention comprises the following 5 fluorophores: fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), cyanine 5 (Cy5) and cyanine 7 (Cy7).

Another preferred combination of fluorophores of the present invention comprises the following 6 fluorophores: fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), Bodipy 630/650, cyanine 5 (Cy5) and cyanine 7 (Cy7).

Another preferred combination of fluorophores of the present invention comprises the following 6 fluorophores: fluorescein isothiocyanate (FITC), Texas Red (TR), cyanine 3 (Cy3), Bodipy 630/650 and cyanine 5 (Cy5).

The combinations of fluorophores according to the invention may be included in a multicolor FISH diagnostic kit.

The combinations of fluorophores according to the invention may be used to label an entity chosen from polypeptides, antibodies, nucleic acids, phospholipids, fatty acids, sterol derivatives, membranes, organelles and biological macromolecules.

In addition, the invention relates to a combination of fluorophores combined with a pair of optical filters chosen from:

a. the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm combined with an excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical),

b. the fluorophore Cy3 having a maximum absorption wavelength of 554 nm

cells were then synchronized by adding methotrexate (10 μM) for 17 hours and then rinsed and recultured in the presence of 5-bromodeoxyuridine (BrdU) (0.1 mM) for 6 hours. After the action of colchicine (1 mg/ml) for 15 min, the disruption of the cells was obtained by resuspension in a hypotonic KCl solution (75 mM). The chromosomes were fixed in a methanol/acetic acid mixture (3 vol/1 vol) and one to two drops of cellular suspension were spread on each slide. The slides, dried at room temperature for 2 to 3 days, were then stored at -20°C for several months.

REFERENCES

Cherif D., Julier C., Delattre O., Derre J., Lathrop GM., Berger R., (1990). Simultaneous localization of cosmids and chromosome R-banding by fluorescence microscopy: Application to regional mapping of chromosome 11. Proc. Natl. Acad. Sci. USA 87, 6639.

Dutrillaux B., Lejeune J. (1971). Sur une nouvelle technique d'analyse du caryotype humain (On a new technique for analyzing human karyotype). C.R. Acad. Sci. 272, 2638.

Johnson G.D., De Nogueira C., Aranjo J.G.M. (1981). A simple method for reducing the fading of immunofluorescence during microscopy. J. Immunol. Methods 43, 349.

Korenberg J.R., Rikowski M.C. (1988). Human genome organization: Alu, Lines and the molecular structure of metaphase chromosome bands. Cell 53, 391.

Ledbetter S.A.,Garcia-Heras J., Ledbetter D.H. (1990). "PCR-karyotype" of human chromosomes in somatic cell hybrids. Genomics 8, 614.

Lichter P., Ledbetter S.A., Ledbetter D.H., Ward D.C. (1990). Fluorescence *in situ* hybridization with Alu and L1 polymerase chain reaction probes for rapid characterization of human chromosomes in hybrid cell lines. Proc. Natl. Acad. Sci. USA 87, 6634.

Pinkel D., Straume T., Gray J.W. (1996). Cytogenetic analysis using quantitative, high sensitivity fluorescence hybridization. Proc. Natl. Acad. Sci. USA 83, 2934.

Richard P Haugland, 1996, Molecular Probes, MTZ Spence Ed. « Handbook of fluorescent probes and research chemicals »

Romana S.P., Tachdjian G., Druart L., Cohen D., Berger R., Cherif D. (1993). A simple method for prenatal diagnosis of trisomy 21 on uncultured amniocytes. Eur. J. Hum. Genet. 1, 245.

SabileA., Poras I., Cherif D., Goodfellow P., Avner P. (1997). Isolation of monochromosomal hybrid for mouse chromosomes 3,6, 10, 12, 14 and 18. Mammalian Genome 8, 81.

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Frank C. Eisenschenk, Ph.D., Patent Attorney

AMENDMENT UNDER 37 C.F.R. § 1.111
Examining Group 1637
Patent Application
Docket No. G-052US02PCT
Serial No. 09/807,507

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Kenneth R. Horlick, Ph.D.
Art Unit	:	1637
Applicant	:	Dorra Cherif
Serial No.	:	09/807,507
Filed	:	April 13, 2001
Conf. No.	:	9446
For	:	Fluorescent Probes for Chromosome Painting

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

AMENDMENT UNDER 37 C.F.R. § 1.111

Sir:

In response to the Office Action dated April 4, 2005, please amend the above-identified patent application as follows:

In the Claims

1-10 (canceled)

11 (currently amended). A method of producing probes intended for labeling human chromosomes comprising mixing first amplification products and second amplification products obtained by two IRS-PCR amplifications from said chromosomes and labeling said amplification products with one or more fluorophore, wherein said first amplification products are obtained using PCR primers specific for ~~the~~ Alu and LINE DNA sequences and said, ~~said~~ second amplification products are obtained using PCR primers specific ~~for the~~for Alu DNA sequences and said amplification products are labeled with:

(a) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical);

(b) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical); and

(c) least one fluorophore, absorption filter, and emission filter are selected from the group consisting of:

(i) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm is coupled with the excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical);

(ii) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emissions wavelength of 670 nm combined

with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical);

(iii) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emissions wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical);

(iv) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical); and

(v) the fluorophore Bodipv 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm used with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650EFLP type (Omega Optical).

12-26 (canceled).

27 (currently amended). A method of identifying human chromosomes comprising performing a multicolor FISH analysis using a plurality of probes and hybridizing one or more human chromosomes with said plurality of probes, said plurality of probes comprising a set of DNA segments which are more represented in certain chromosome bands and which are obtained by IRS-PCR amplification from said chromosomes ~~with the aid of~~using primers specific ~~for the~~ for Alu and LINE DNA sequences and said probes are labeled with one or more fluorophore, each of said one or more fluorophore having a specific absorption and emission wavelength, wherein each of said one or more fluorophore is used with a pair of optical filters, one for absorption and one for emission, and wherein said fluorophore and pairs of filters are selected from the group consisting of:

(a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm used with the excitation filter of the

490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical);

(b) the fluorophore Cy3 having maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm used with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical);

(c) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm used with a excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical);

(d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emissions wavelength of 670 nm used with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical);

(e) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm used with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical); and

(f) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm used with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650EFLP type (Omega Optical).

28 (canceled).

29 (currently amended). The method of claim 27 ~~claim 28~~, wherein the optical filters exhibit the following qualities:

they are of the 6-cavity type;

they have an ADI of 0°;

they have a tolerance $\lambda_o \pm 20\%$ of FWHM;

they have a tolerance on FWHM of $\pm$ 20% of FWHM;

they have an OD5 out-of-passband rejection of UV at 1200 nm;

they have a transmission curve $T \geq 50\%$ at λ_o.

30 (previously presented). The method of claim 29, wherein the optical filters exhibit, in addition, the following characteristics:

they have a centered useful diameter greater than 21 nm;

they have a thickness $\leq$ 7 mm.

31 (previously presented). The method of claim 27, wherein said multicolor FISH is a karyotype analysis.

32 (previously presented). The method of claim 31, wherein said karyotype analysis is performed to detect chromosome rearrangements.

33-36 (canceled).

37 (currently amended). A kit comprising at least one fluorophore having a specific absorption and emission wavelength, said kit further comprising at least one pair of optical filters, said pair of optical filters comprising one absorption filter for detecting signals at said absorption wavelength and one emission filter for detecting signals at said emission wavelength, wherein said at least one fluorophore, absorption filter, and emission filter are selected from the group consisting of:

(a) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm is coupled with the excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical);

(b) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the

546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical);

(c) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical);

(d) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emissions wavelength of 670 nm combined with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical);

~~(e) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emissions wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical);~~

~~(f)~~(e) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical); and

~~(g)~~(f) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm used with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650EFLP type (Omega Optical).

38-40 (canceled).

41 (new). The kit according to claim 37, wherein said kit further comprises the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emissions wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical).

42 (new). The kit according to claim 37, wherein said kit comprises:

(a) the fluorophore Cy3 having a maximum absorption wavelength of 554 nm and a maximum emission wavelength of 568 nm combined with an excitation filter of the 546DF10 type (Omega Optical) and with an emission filter of the 570DF10 type (Omega Optical);

(b) the fluorophore TR having a maximum absorption wavelength of 593 nm and a maximum emission wavelength of 613 nm combined with an excitation filter of the 590DF10 type (Omega Optical) and with an emission filter of the 615DF10 type (Omega Optical); and

(c) least one fluorophore, absorption filter, and emission filter are selected from the group consisting of:

(i) the fluorophore FITC having a maximum absorption wavelength of 494 nm and a maximum emission wavelength of 517 nm is coupled with the excitation filter of the 490DF30 type (Omega Optical) and with an emission filter of the 530DF30 type (Omega Optical);

(ii) the fluorophore Cy5 having a maximum absorption wavelength of 652 nm and a maximum emissions wavelength of 670 nm combined with an excitation filter of the 650DF20 type (Omega Optical) and with an emission filter of the 670DF10 type (Omega Optical);

(iii) the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emissions wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical);

(iv) the fluorophore Cy5.5 having a maximum absorption wavelength of 675 nm and a maximum emission wavelength of 694 nm combined with an excitation filter of the 680DF20 type (Omega Optical) and with an emission filter of the 700EFLP type (Omega Optical);

(v) the fluorophore Bodipy 630/650 having a maximum absorption wavelength of 632 nm and a maximum emission wavelength of 658 nm used with an excitation filter of the 630DF20 type (Omega Optical) and with an emission filter of the 650EFLP type (Omega Optical).

43 (new). The kit according to claim 37, wherein said kit comprises at least two of said fluorophores and at least two pair of said optical filters.

44 (new). The kit according to claim 37, wherein said kit comprises at least three of said fluorophores and at least three pair of said optical filters.

45 (new). The kit according to claim 37, wherein said kit comprises at least four of said fluorophores and at least four pair of said optical filters.

46 (new). The kit according to claim 37, wherein said kit comprises at least five of said fluorophores and at least five pair of said optical filters.

47 (new). The kit according to claim 43, wherein said kit further comprises the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emissions wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical).

48 (new). The kit according to claim 44, wherein said kit further comprises the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emissions wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical).

49 (new). The kit according to claim 45, wherein said kit further comprises the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emissions

wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical).

50 (new). The kit according to claim 46, wherein said kit further comprises the fluorophore Cy7 having a maximum absorption wavelength of 743 nm and a maximum emissions wavelength of 767 nm combined with an excitation filter of the 740DF25 type (Omega Optical) and with an emission filter of the 780EFLP type (Omega Optical).

Remarks

Claims 11, 27-32, and 37 are pending in the subject application. By this Amendment, Applicant has canceled claims 28 and 33-36, amended claims 11, 27, 29, and 37, and added new claims 41-50. Support for the amendments and new claims can be found throughout the subject specification and in the claims as originally filed (see, for example, page 7, lines 21-31; page 9, line 17 through page 16, line 21). Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 11, 27, 29-32, 37, and 41-50 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested.

As an initial matter, the Examiner indicates that the Dutrillaux *et al.* reference cited as "M" on the Information Disclosure Statement filed May 29, 2005 has not been considered because it is not in English. In fulfillment of Applicant's duty to provide an explanation of the relevance of foreign language document, Applicant would like to bring to the Examiner's attention a Supplemental Information Disclosure Statement which is being submitted in conjunction with the filing of this Amendment. Applicant respectfully requests that the reference be considered and made of record by the Examiner in the subject application.

Claims 11 and 27-32 are rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Applicant has amended claims 11 and 27 to correct the antecedent basis issue identified for claim 11 in the Office Action and to clarify the primers and their use in claims 27 and 29-32. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, is respectfully requested.

Claim 11 is rejected under 35 U.S.C. § 102(b) as anticipated by Wilgenbus *et al.* (1995). The Office Action argues that Wilgenbus *et al.* anticipate the claimed invention. As the Patent Office is aware, a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a prior art reference. *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). It is respectfully submitted that Wilgenbus *et al.* (1995) fail to anticipate the claims as the reference fails to teach the labeling of primers with the recited fluorophores. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 102(b) is respectfully requested.

Claim 37 is rejected under 35 U.S.C. § 103(a) as obvious over Speicher *et al.* (1996) in view of Siciliano *et al.* (U.S. Patent No. 5,538,869). The Office Action argues that Speicher *et al.* teach a combination of the fluorophore Cy7, the excitation filter Omega 740DF25 and the emission filter Omega 780EFLP. The Office Action also indicates that Speicher *et al.* fail to teach a kit. The Office Action argues that Siciliano *et al.* renders the claimed kit obvious as it teaches kits and because it was conventional in the art at the time of the invention to package together reagents into a kit for the convenience of practicing methods that required the reagents. Applicant traverses.

To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 U.S.P.Q. 580 (C.C.P.A. 1974). Applicant respectfully submits that the combination of cited references fail to establish a *prima facie* case of obviousness for the claimed invention as it fails to teach or suggest all the limitations recited within the claims. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

Claims 27-32 are rejected under 35 U.S.C. §103(a) as obvious over Lichter *et al.* (1990), Lengauer *et al.* (1990), or Wilgenbus *et al.* (1995) in view of Speicher *et al.* (1996). The Office Action states that the Wilgenbus *et al.* reference teaches a method comprising mixing first and second amplification products obtained from two IRS-PCR amplification reactions from chromosomes, wherein the first amplification products are obtained using Alu- and LINE-specific primers and the second amplification products are obtained using Alu-specific primers. The Office Action indicates that the Speicher *et al.* reference teaches a combination of the fluorophore Cy7, the excitation filter Omega 740DF25, and the emission filter Omega 780EFLP. The Office Action further indicates that the Lichter *et al.*, Lengauer *et al.*, and Wilgenbus *et al.* references teach using probes obtained by IRS-PCR amplification using primers specific for Alu and LINE DNA sequences in FISH analysis. Applicant respectfully traverses.

As indicated above, all the claim limitations must be taught or suggested by the prior art in order to establish the *prima facie* obviousness of a claimed invention. Applicant, again, respectfully submits that the cited combination of references fails to render the claimed invention obvious as they fail to teach or suggest the recited fluorophore and filter combinations. As the combination of references fail to teach or suggest the recited claim limitations, it is respectfully submitted that a

prima facie case of obviousness has not been established and withdrawal of the rejection under 35 U.S.C. §103(a) is respectfully requested.

Claim 11 is rejected under the doctrine of "obviousness-type" double patenting over claim 1 of U.S. Patent No. 6,562,959 and claim 37 is rejected under the doctrine of "obviousness-type" double patenting over claim 10 of U.S. Patent No. 6,562,959. Additionally, claims 11 and 27-32 have been provisionally rejected under the doctrine of "obviousness-type" double patenting over the claims of co-pending Application No. 10/251,699 (now U.S. Patent No. 6,905,828). Applicants have concurrently filed a Terminal Disclaimer in this matter and respectfully submit that these rejections are now moot. Accordingly, reconsideration and withdrawal of the rejections is respectfully requested.

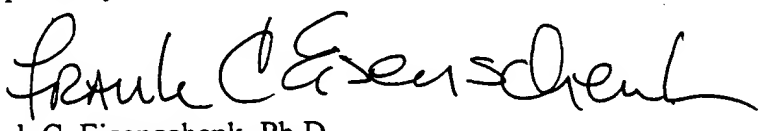
It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicant's agreement with or acquiescence in the Examiner's position. Applicant expressly reserves the right to pursue the invention(s) disclosed in the subject application, including any subject matter canceled or not pursued during prosecution of the subject application, in a related application.

In view of the foregoing remarks and amendments to the claims, Applicant believes that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicant invites the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



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